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George Fedak
Agriculture Canada

L. O'Donoughue
Agriculture Canada

K. C. Armstrong
Agriculture Canada

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George Fedak, L. O'Donoughue, K.C. Armstrong

Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada, K1A OC6

ABSTRACT

The steps involved in the transfer of alien genetic variation will be outlined and the impact of recent technologies on improving the efficiency of the process will be discussed. The selection of parents is the first step; it is critically important that each selection be carefully screened for maximum expression of the desired trait. The crossing process is becoming increasingly more efficient with improved efficiency of growth regulators and embryo rescue media. Doubled haploid methods are being used to facilitate the production of chromosome addition lines. Molecular methods such as RFLPs, RAPDs, chromosome banding, and *in situ* hybridization add an increased level of resolution to the identification of chromosome additions and the monitoring of introgressed chromosome segments. Emerging technologies such as monocot transformation, chromosome-specific libraries, and transposon tagging may soon replace some of the traditional methods of gene transfer.

INTRODUCTION

Plant breeding effort is directed at the production of cultivars showing improvement in yield plus improvements in any number of agronomic traits is an ongoing and upwardly incremental process. Improved cultivars of every crop are being released at a steady pace. All such cultivars are regarded as elite germplasm. The major prerequisite for the plant breeding process is a continued supply of genetic variability. For most traits, ample variability exists in the primary gene pool.

The first consequence of ranging into the secondary gene pool obtain desirable agronomic traits is dilution of the elite germplasm. Repeated backcrossing to the elite recurrent parent becomes necessary to restore the original cultivar. Therefore wide crossing for purposes of gene transfer is done as a last resort when the variability for a particular trait is exhausted or non-existent in the primary gene pool. Examples of traits that show low variability in primary gene pools are resistance to BYDV, streak mosaic, *Fusarium* head blight in wheat, and net blotch-root rot in barley. Other traits that are being sought in alien species with the objective to transfer to wheat are perennial habit, resistance to leaf stem and stripe rust, curl mite resistance, increased protein content, yellow semolina color, and apomixis. In addition there is limited variability and ample room for improvement to tolerance to abiotic stresses such as cold, drought, heat, and salinity in wheat, barley and rye. There are some 400 species and 25 genera in the Triticeae tribe and the scope for intergeneric hybridization is immense. Numerous intergeneric hybrids within the tribe have already been made with wheat (Miller, 1987; Pienaar, 1990) and barley (Fedak, 1992).

BREEDING

Germplasm - parents

The first and probably single most important criterion determining the success of a wide crossing program is the critical screening of the parental material. Sources of alien germplasm are the genebank networks and the variability in the wild that can still be collected. In the case of the latter there is considerable knowledge available on the natural distribution of Triticeae species plus the types of stresses they encounter. This offers some clues as to the geographical distribution of the traits in question. Natural selection pressures seem to have been effective in concentrating certain types of variability.

Whatever the source of variability, whether botanical collection or gene bank, every accession of every species needs to be thoroughly screened to ensure the maximum expression of the trait in question (Sharma *et al.*, 1984). This trait may be polygenic in inheritance or the source could be heterozygous and thus complicate the transfer procedure. Problems of trait expression are often encountered in the progeny of wide crosses so every precaution should be taken in selecting the best possible parents.

Selection for abiotic stress resistance is usually done in *in vitro* cultures while selection for disease resistance has been done by exposure to the organism itself. Molecular tags and known sequences for specific genes will be useful in the future. For traits such as storage protein genes, cloned probes have become powerful tools for screening parents for variability, for example, hordein genes in barley (Bunce *et al.*, 1986), gliadin genes in bread wheat (Bartels *et al.*, 1986), and gliadin and glutenin PCR probes in wheat (D'Ovidio *et al.*, 1992).

Crossing

It is now believed by some researchers involved in intergeneric hybridization within the Triticeae that hybrids can be made between virtually any two species within the tribe, Most species can be crossed onto wheat in particular and many onto barley and rye. The most important condition is that sufficient numbers of accessions of a particular species be evaluated. The other factors involved are the pre and post-pollination applications of growth regulators. For example, it was shown that whereas the post-pollination application of GA_3 to barley enhances seed set and embryo differentiation, 2,4-D is more effective than GA_3 on wheat following wheat x maize pollination for haploid production (Fedak *et al.*, 1994). Embryo rescue media are becoming more complex but also more effective. In some cases immature ovules at two days post pollination can be rescued on the more complex media (Comeau *et al.*, 1992). The nurse culture technique (Kruse, 1973), though quite labor intensive, was effective in rescuing hybrid embryos from *Hordeum* x *Secale* crosses (Fedak, 1979). Employing such techniques a total of 300 intergeneric hybrids in *Hordeum* have been reported (see Fedak, 1992 for summary) and probably an equal number involving wheat (Pienaar, 1990).

Genome analysis

The relationship between species (and to a certain extent between genomes) in the Triticeae has been the subject of decades of ongoing study. Species relationships *per se* can be deduced from analysis of the parental species prior to making hybrids by employing techniques such as C banding (Linde-Laursen *et al.*, 1992), N banding (Gecheff *et al.*, 1994), and isozyme analysis (Hart, 1987), RFLP analysis using repetitive sequence probes (Molnar *et al.*, 1989; Gupta *et al.*, 1989; Appels *et al.*, 1989). Techniques have also been developed to conduct sequential banding and *in situ* hybridization for mapping of euchromatic and heterochromatic regions of wheat chromosomes (Jiang and Gill, 1993b).

The extent of meiotic chromosome pairing will provide indications of homoeology between parental genomes in the hybrid for another estimate of genome and hence species relationships. In hybrids involving crop and wild species, meiotic data also provide estimates of species relationships. The amount of chromosome pairing is an indication of the amount of recombination that might be expected and hence potential gene transfer. In hybrids within the Triticeae especially involving polyploid species, it has been virtually impossible with conventional staining methods to distinguish between autosyndetic and allosyndetic pairing. The concept of *in situ* hybridization using fluorescent- labelled total genomic DNA as a probe with genome blocking has only recently been reported as a means of identifying component genomes at meiosis (King, 1993). Fluorescent *in situ* hybridization using labelled total genomic DNA of one species and blocking DNA from the other species on somatic chromosome preparations has been employed for several years to distinguish firstly, component genomes in a hybrid and secondly detect any piece of introgressed alien chromatin such as wheat-alien addition substitution and translocation lines (for review see Jiang and Gill, 1994). Individual genomes and introgressed segments can be differentiated by direct labelling of component and DNA (Anamthawat-Jonsson and Reada, 1995), as a more efficient alternative to genome blocking.

Chromosome doubling

The old standard methods of chromosome doubling through the use of spindle fibre suppressants such as colchicine and methotrexate are still in use today. Chromosome doubling of intergenomic hybrid plants regenerated from callus cultures has been reported (Fedak and Grainger, 1986; Wang, 1992). The success rate in producing amphiploids from intergeneric hybrids in the Triticeae has generally been quite low. In hybrids involving *Hordeum* species or cultivars the success rate has been negligible although there are indications that colchicine response is genotype dependent as evidenced by the amphiploid obtained from the *H. californicum* x Chinese Spring hybrid (Fedak, 1987).

Backcrossing

The ideal method of achieving gene transfer from an alien species into a crop plant is to backcross the hybrid or amphiploid to the crop plant until the complete series of addition lines is produced. Even if chromosome doubling treatments on the hybrid are not effective, it is usually possible to backcross onto the hybrid, particularly hybrids involving wheat as one of the parents. This is usually mediated by the production of restitution nuclei in the hybrid. In some cases, tens of thousands of florets had to be pollinated to obtain a backcross on the hybrid involving wheat x *Elymus angustus* (A. Plourde, p.c.). Negligible success has been reported in trying to obtain backcrosses with any hybrids involving barley cultivars or *Hordeum* species. Partial amphiploids (with 2n=56 and having one alien genome) are one, albeit rare, product of backcrossing

wheat onto wheat-6x *Thinopyrum* hybrids (Cauderon, 1966; Pienaar, 1988). Otherwise backcrossing is continued until the entire series of alien addition lines are produced in the crop plant background. Several effective methods of rapidly obtaining disomic addition lines involve the crossing of a BC_1 plant back onto an amphiploid (Lukashewski, 1988) or by producing haploids with an additional chromosome, then doubling the chromosome number (Fedak, unpublished). Such a situation was encountered in attempting to produce disomic addition lines of *H. californicum* in a Chinese Spring background. No disomic additions were recovered in the selfed progenies of monosomic additions presumably because of a lack of transmission of the monosome through the pollen.

Even though barley tolerates trisomy, telotrisomy, triploidy, tetraploidy and a few cases of tetrasomy, it does not appear to tolerate the addition of alien chromosomes or genomes. For example, triploid hybrids containing 14 barley and 7 rye chromosomes have been produced (Fedak and Nakamura, 1982), but such plants were sterile and further backcrosses to barley to produce rye addition lines were not successful. Similarly, numerous interspecific hybrids were produced with barley (Jacobsen and Bothmer, 1981), but with the exception of hybrids with *H. spontaneum* x *H. bulbosum*, backcrossing with other barley interspecific hybrids has not been successful.

Identification of critical chromosome additions

The identification of addition lines involves the determination of the homoeologous relationship of the additional chromosome and also the identification of the addition line carrying the trait in question. The homoeology of the added chromosome can be determined by employing various markers with known chromosome locations such as isozymes (William and Kazi, 1993; Hart, 1987; Forster *et al.*, 1987), C banding (Dhaliwal *et al.*, 1990), RFLP markers (Anderson *et al.*, 1992; Sharp *et al.*, 1989), RAPD markers (Penner *et al.*, 1993), STS markers (Talbert *et al.*, 1994), or specific banding patterns (Gill, 1987; Jiang and Gill, 1993b).

Some examples of identification of addition lines carrying specific traits include: chromosome 7 addition of *Th. intermedium* provides resistance to BYDV (Brittel *et al.*, 1988), additions of chromosomes of *Th. elongatum* provides salinity tolerance (Dvorak *et al.*, 1985), chromosome 5J of *Th. junceum* provides salinity tolerance (Forster *et al.*, 1988), addition lines of chromosome $1H^{ch}$ of *Hordeum chilense* added to wheat provide resistance to the root-knot nematode (Perron-Dedryver et al., 1990) and *E. ciliaris* chromosomes $1S^c$ or $1Y^c$ restore fertility to alloplasmic euploid wheat with *E. ciliaris* cytoplasm (Jiang *et al.*, 1993). A compendium of wheat-*Thinopyrum* addition, substitution, and translocation lines has been published (Shepard and Islam, 1988).

Induction of recombination

Although all the species within the Triticeae originated from a common ancestor and share basic genomes that may or may not have undergone chromosome rearrangements, chromosome pairing may be under genetic control so that homoeology is not fully expressed. The best known example is the *Ph* locus of wheat that restricts chromosome pairing to strictly bivalent formation (Sears, 1976) so that any homoeologous pairing must be induced. A *Ph* mutant has also been isolated in durum wheat (Georgi, 1978). There are several standard methods of induction of homoeologous chromosome pairing in wheat. In wheat itself there are aneuploids such as nulli 5B and mutations at the *Ph* locus (Sears, 1984) that permit pairing of wheat with homoeologous alien chromosomes. Sears (1973) was the first to use the above tools to transfer leaf rust resistance from a *Th. ponticum* chromosome 7 substitution to chromosome 7D of wheat. Numerous other researchers were able to use the same tools to induce meiotic crossing-over and transfer of traits from wheat to alien chromosomes (See Pienaar, 1990 for summary of wheat-*Thinopyrum* transfers).

The genomes of certain *Aegilops* species such as *Ae. speltoides* are known to suppress the *Ph* system of hexaploid wheat and thus permit homoeologous pairing. By this means translocations between *Th. intermedium* and wheat chromosomes were induced (Ortiz *et al.*, 1986).

Radiation with X or gamma-rays of seed or poffen of hybrids, partial amphiploids, and addition or substitution lines have induced recombinations and gene transfers. Numerous examples are listed in the review by Pienaar (1990). A more recent method for recombination induction involves the production, extended proliferation, and maintenance of callus induced from vegetative or reproductive parts of hybrids or aneuploid plants. Plants regenerated from such callus were shown to contain numerous chromosome translocations (Fedak, 1990).

Spontaneous translocations between wheat and alien chromosomes do occur at a low frequency at meiosis and several examples of transfers of useful genes have been documented (See Pienaar, 1990 for summary).

Screening of progenies

The induction of interchanges produces vast numbers of critical and non critical chromosome translocations. The usual large numbers of derivatives must be screened to isolate those carrying the desired gene(s). The usual approach is to inoculate with the specific pathogen, expose to a strong selection pressure, or electrophoretically analyze progeny for a particular value-added trait. Rapid screening methods are continually being devised to expedite this process.

Molecular tags are rapidly being developed for numerous agronomic traits, particularly those that are

simply inherited, eg., disease resistance loci (Hinze *et al.*, 1991; Penner *et al.*, 1993), biochemical loci (Kilian *et al.*, 1994), and value-added traits (Reddy and Appels, 1993). Such markers can rapidly be developed as particular needs arise. Desirable genotypes can be selected based on molecular markers rather than selecting for the trait itself. The advantage of such markers is the ability to screen for traits that are recessive, difficult to score, obscured by other traits.

Linkage maps have been assembled from RFLP markers in various crop plants, including wheat (Gale *et al.*, 1990; Anderson *et al.*, 1992), barley (Huen *et al.*, 1991; Graner *et al.*, 1991; Kleinhofs *et al.*, 1993), and rye (Wang *et al.*, 1991). These are being combined with isozyme, biochemical, and morphological markers in order to maximize coverage. Such maps are becoming a valuable tool in screening and mapping polygenic traits as quantitative trait loci (QTL) (Hayes *et al.*, 1993). Such tags will permit the monitoring of such loci during their transfer and integration. RAPD markers are a cheaper and simpler method of providing gene tags. They function by amplifying a sequence that appears as an electrophoretic band, that is closely linked to a gene in question (Penner *et al.*, 1993; Procunier *et al.*, 1994). Alternatively, the primer can be an actual base sequence of the transferred chromosome segment or particular gene. An example of the latter is a sequence of a virus coat protein gene that can be used on slot blots to detect the presence of BYDV in recombined progenies (Ouellette and Fedak, unpublished). Another example of a rapid screening technique is the use of monoclonal antibodies to screen for various metabolites such as the toxin produced by *Fusarium graminearum* (Sinha and Savard, unpublished).

Detection of alien chromatin

The objective in transferring traits from alien sources into crop plant chromosomes is to transfer the trait with minimal amounts of additional chromatin. With traditional staining methods it has been virtually impossible to detect the amount of alien chromatin that was translocated. The *in situ* hybridization techniques applied to somatic chromosomes of derivatives have effectively identified the integrated chromatin.

For example, the resistance to Hessian fly located on a 6RL rye telocentric chromosome addition was transferred to a terminal site on wheat chromosomes 6BS and 4BS following pollen irradiation (Mukai *et al.*, 1993) and to an interstitial location on wheat chromosome 4BS. This was achieved by sequential C banding and *in situ* hybridization using highly repetitive rye DNA probes. The size of the translocated segment was revealed in each case.

In similar fashion, the segment of *Th. intermedium* chromosome 7A carrying the leaf rust resistant gene *Lr38* was found to be translocated to wheat chromosomes 2AL, 5AS, 1DL, 3DS and 6DL in the different lines that were analyzed (Friebe *et al.*, 1993). The translocations were induced by Co^{60} treatment of a wheat-*Thinopyrum* addition line. Similarly a segment of a chromosome carrying wheat streak mosaic virus resistance was transferred from a wheat - *A. elongatum* disomic substitution line carrying chromosomal Ae-1L onto wheat chromosome 4DL (Jiang *et al.*, 1993).

The use of *in situ* hybridization adds a greater degree of resolution to cytogenetic studies. For example it was shown that what had previously been assumed to be a Robertsonian translocation based on ditelo analysis of a translocation line (Whelan and Hart, 1989) actually involved a proximal portion of the opposite arm (Kim *et al.*, 1992, 1993a).

A study of two wheat derivatives with radiation induced leaf rust resistance from *Th. ponticum* (Kim *et al.*, 1993b) showed that the lengths of the translocated segments were vastly different and that intercrosses between the lines and further *in situ* hybridization would be warranted to reduce the size of the translocated segment. It was also shown that considerable discrepancy existed between the physical map based on *in situ* hybridization patterns and the linkage map based on RFLP markers.

Current activities

There are a number of ongoing research activities of a genetic-cytogenetic nature but with a molecular base. These are going to provide more precise basic information about genetic-cytogenetic nature of relationships of Triticeae species, genome structure and evolution, and chromosome synteny. Additional techniques will facilitate gene flow from constituent members of the tribe into crop plant members.

Genomic *in situ* hybridization combined with RFLP maps of the major crops will provide better indications of genome structures within these crops in terms of inter and intragenomic translocations at the diploid parent level and derived polyploid level. An indication of such findings is the extensive interchromosomal translocations already detected in rye (Liu *et al.*, 1992). This will facilitate studies of species and crop plant evolution. The comparative RFLP mapping that is already underway will broaden the knowledge of syntenic relationships across species and crop plants. Since gene order is conserved across many species the location of a gene in a domestic species may be used to predict the location in a wild species. As gene tagging and map-based cloning techniques develop, this information will be useful especially for species where sexual hybridization is difficult.

Over the past year and following considerable effort, several labs have now reported the stable transformation of both wheat (Weeks *et al.*, 1993; Bilang *et al.*, 1993; Becker *et al.*, 1994; Nehra *et al.*, 1994) and barley (Wan

and Lemaun, 1994). These achievements can probably be regarded as the most significant achievements in crop plant genetics in the past few decades. This will open up the possibilities of gene flow from any living organism into crop plants. The genome of rice may become the major source to circumvent gene isolation from the large and complex genomes of wheat and barley. The production of chromosome- specific libraries through microdissection (Albani *et al.*, 1993) will produce saturated RFLP maps for possible applications of map-based cloning.

Gene tagging with RFLPs and RAPDs will provide molecular markers for ever increasing numbers of genes. The RFLP markers are being converted to STS for PCR use while RAPDs are being converted to SCARS to provide extended applications over genotypes. The gene tags will permit the pyramiding of various gene combinations.

Traits such as tolerance to abiotic stresses, levels of value-added traits, and yield and its components are generally regarded to be controlled by polygenic systems. The production of RFLP maps of ever-increasing density will permit for the first time a better understanding of the QTL phenomenon, the chromosomal location of these factors, and a method of monitoring their manipulation.

With transformation now a reality, the incorporation of transposable elements into wheat and barley should be a possibility and thus an alternate method of gene tagging. Map-based cloning of genes from barley and wheat will undoubtedly be difficult because of the large genome size and large proportion of highly repetitive sequences, so rice may be an alternate source of genes for transformation of these two crops. Rice carries a much smaller genome that has been well mapped. The above are a few examples of techniques that are already being applied. New methods are bound to arise from the above that will be quicker, cheaper, and more discriminatory.

Although the technologies mentioned above have all been reported, they are still not routinely applied. RFLP maps have identified location of QTLs and quantitative loci to chromosome regions or linkage groups, for map-based cloning markers that flank the gene at distances of about 1 centimorgan are required. Very few such tight linkages have been reported. Transformation and stable integration of traits has been reported for all the major cereal crops, the frequencies are low and only a small proportion of transformations are stable. "Fine-tuning" of the above technologies is essential before they become routine.

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